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### **Title**

Light Regulation of Alternative Pre-mRNA Splicing in Plants.

### **Permalink**

<https://escholarship.org/uc/item/572943kr>

### **Journal**

Photochemistry and photobiology, 93(1)

### **ISSN**

0031-8655

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### **Publication Date**

2017

### **DOI**

10.1111/php.12680

Peer reviewed

## Invited Review

# Light Regulation of Alternative Pre-mRNA Splicing in Plants<sup>†</sup>

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Received 31 July 2016, accepted 20 November 2016, DOI: 10.1111/php.12680

## ABSTRACT

Alternative splicing (AS) is a major post-transcriptional mechanism to enhance the diversity of proteome in response to environmental signals. Among the numerous external signals perceived by plants, light is the most crucial one. Plants utilize complex photoreceptor signaling networks to sense different light conditions and adjust their growth and development accordingly. Although light-mediated gene expression has been widely investigated, little is known regarding the mechanism of light affecting AS to modulate mRNA at the post-transcriptional level. In this minireview, we summarize current progresses on how light affects AS, and how sensory photoreceptors and retrograde signaling pathways may coordinately regulate AS of pre-mRNAs. In addition, we also discuss the possibility that AS of the mRNAs encoding photoreceptors may be involved in feedback control of AS. We hypothesize that light regulation of the expression and activity of splicing factors would be a major mechanism of light-mediated AS. The combination of genetic study and high-throughput analyses of AS and splicing complexes in response to light is likely to further advance our understanding of the molecular mechanisms underlying light control of AS and plant development.

## INTRODUCTION

In eukaryotes, introns usually need to be spliced out to form mature mRNAs in the nucleus (1). The splicing machinery utilizes different splice sites to produce two or more isoforms at the same locus (2,3). The discovery of alternative splicing (AS) is a key milestone for understanding the post-transcriptional regulation (4–7). Early studies based on EST and full-length cDNA demonstrate that AS presents a larger proportion of multi-intron genes in plants (8). Recent genomewide studies based on next-generation sequencing confirm that a large number of genes are regulated by AS, including more than 60% of the intron-containing genes in both *Arabidopsis* and *Glycine max* (9–11), approximately 33% of the annotated genes in *Oryza sativa* (12), and at least 56% of the genes in *Zea mays* (12,13).

Alternative splicing can be classified into four major types: intron retention, exon skipping, alternative donor and acceptor sites (9,14). Intron retention, as the most prevalent type of AS in plants, often produces splicing isoforms containing premature termination codon (PTC) that can be targeted by nonsense-mediated mRNA decay (NMD) to regulate the transcriptional level of functional transcription (8–10,15,16). Furthermore, splicing isoforms with PTC can also produce truncated proteins to regulate the full-length functional proteins (17–20). Other types of AS including exon skipping, alternative donor and acceptor sites produce various proteins with distinct subcellular localization, stability and binding properties by in-frame deletion or addition of alternative domains (3,14,21,22).

Alternative splicing enhances the adaptability of plants in response to environmental alterations (23). Emerging studies have revealed that AS plays an essential role in light response by regulating core-clock genes, including *RVE8*, *JMJD5*, *LHY*, *TIC* and *CKB3* (24–26). Meanwhile, light quality and quantity can regulate AS of numerous plant genes (24,27,28). These findings enhance our understanding of how plants adapt themselves to track light oscillations via AS-mediated regulation. However, the mechanisms of light regulation of AS are still not fully understood. Here, we summarize the latest findings of light-mediated AS regulation and propose the possible mechanisms of AS regulation in response to light.

## Transcriptome-wide regulation of AS in response to light

Due to technical limitations, only a subset of AS regulated by light has been revealed by earlier studies (29,30). For example, the first study reports nine genes with differential spliced pattern upon light treatment, including carboxypeptidase, tyrosyl tRNA synthetase transcripts and two serine/arginine-rich (SR) genes using the AS RT-PCR panel (31). Another study shows that splice forms from ten genes displays opposite splicing patterns in the light- vs. dark-treated samples using whole-genome oligonucleotide array (32). Recent development of high-throughput sequencing technologies allows genomewide survey of splicing isoforms (33,34). Three genomewide transcriptome studies using RNA-seq reveal that light affects AS of hundreds to thousands of genes (24,27,28). In *Arabidopsis*, at least 7% of protein-coding genes exhibit AS pattern mediated by phytochrome A (phyA) and phyB, two major molecular species of red/far-red-sensing phytochromes (27). Genomewide study by Wu *et al.* (28) in the moss *Physcomitrella patens* shows 8.4% and 8.9% of AS events in

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<sup>†</sup>This article is part of the special issue highlighting Dr. Aziz Sancar's outstanding contributions to various aspects of the repair of DNA photodamage in honor of his recent Nobel Prize in Chemistry.

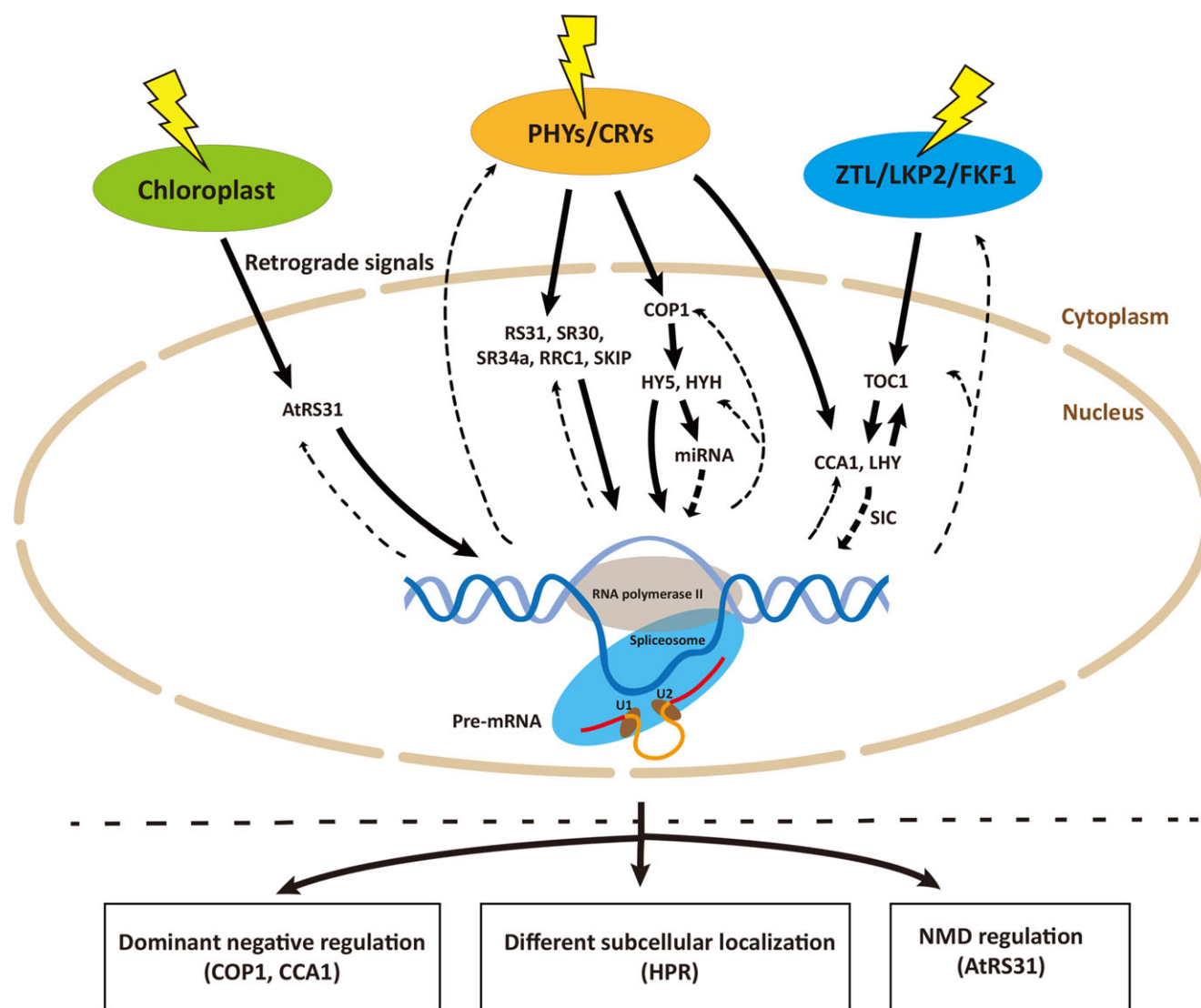
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response to red and blue light, respectively. Most recently, a large-scale transcriptome profiling in *Arabidopsis* estimates different AS patterns in 382 genes upon 2-h pulse of white light in the middle of the night (24). Alternatively, as the most frequent AS events, intron retentions are mainly induced by phytochromes (24,28). These observations suggest that mRNA splicing is directly involved in the photomorphogenic response in plants (Fig. 1). Taking advantage of current whole-transcriptome sequencing strategies, more light-mediated splicing isoforms will be discovered in the future.

### AS mediated by sensory photoreceptors and retrograde signaling pathway

A couple of photosensory receptors, including five phytochromes (phyA–phyE), UV-A/blue light-absorbing receptors such as two phototropins (phot1, phot2), two cryptochromes (cry1, cry2) and

three Zeitlupe proteins (ZTL, FKF1 and LKP2), and the UV-B photoreceptors (UVR8) have been reported (35,36). Phytochrome-dependent change in AS was firstly reported in *Arabidopsis* and demonstrated that 15% of splicing-related genes possess phytochrome-mediated AS after exposing to red light for 1 h (27). Furthermore, phytochrome-deficient mutants in *Physcomitrella patens* cannot response to AS regulation upon light treatment, supporting the involvement of phytochromes in splicing regulation (28). Collectively, these studies suggest that the signaling from sensory photoreceptors, at least phytochromes, is the primary pathway in the light-regulated AS, although the involvement of other photoreceptor in the AS regulation has not been experimentally proved. In contrast, another study reports that light-triggered AS in *AtRS31* is independent of phytochrome and cryptochrome pathways (37). Further study demonstrates that white light-induced splicing alteration in *SR30* is not affected in



**Figure 1.** Model of the regulation of light-mediated AS. Light regulates AS widely via three primary types of sensory photoreceptor pathways (phytochromes, cryptochromes and ZTL/LKP2/FKF1) or retrograde signals from chloroplast. Splicing factors, such as RS31, SR30, SR34a, RRC1 and SKIP, are involved in light-mediated AS regulation, whereas these splicing factors are also under the regulation of AS. Intriguingly, AS of *PHYA*, *CRY2*, *ZTL* and other genes involved in light signaling pathways, such as *COP1*, *HYH*, *CCA1* and *TOC1*, might form a feedback loop in response to light. Solid arrow illustrates genes involved in light signaling pathway to regulate AS. Thick broken arrow represents weak indirect evidence that genes involved in light signaling pathway to regulate AS. Dash arrow indicates genes that are under the regulation of AS. Light-mediated AS has function on dominant negative regulation, different subcellular localization or taking part in NMD pathway.

*phyAB*, *phyABCDE* and *cry1cry2* mutants, suggesting white light may regulate the activity of splicing factors via photosynthetic process (24). In addition, retrograde signals from the chloroplast are also involved in the regulation of AS events (37). These observations support the view that light regulation of AS is not only dependent on photosensory photoreceptors, but also mediated by the photosynthetic process or retrograde signals from the chloroplast (Fig. 1).

### Circadian clock genes regulated by AS in response to light

In the central loop of circadian clock, *TIMING OF CAB EXPRESSION 1 (TOC1)* and *CIRCADIAN CLOCK-ASSOCIATED1 (CCA1)/LATE ELONGATED HYPOCOTYL (LHY)* show reciprocal suppression (38,39). ZTL, together with CCA1 and LHY, can suppress the expression of *TOC1* (40,41). AS is an important mechanism for the regulation of circadian clock-related genes, including *ZTL*, *CCA1*, *LHY* and *TOC1* in *Arabidopsis* (17,42). AS of core-clock genes, such as *LHY*, *RVE8*, *JMJD5*, *TIC* and *CKB3*, is reported to be affected by light (24). It is noteworthy that AS and circadian clock-related genes can be mutually regulated (17,43,44). Clock-related genes, for example, *AtGRP7* and its paralog *AtGRP8* can reciprocally control AS of their transcripts (15,44,45), while splicing factors, such as *SKIP* and *STIPL1*, can regulate the circadian clock in *Arabidopsis* (46,47). Furthermore, more recent studies also pointed out that protein arginine methyltransferase 5 (*PRMT5*) can regulate the AS of core-clock gene *PSEUDO-RESPONSE REGULATOR9 (PRR9)* in *Arabidopsis* (48–50), and the expression of *PRMT5* is clock-regulated (51). These studies strongly indicate that the circadian clock is tightly associated with the regulation of AS.

### Mechanism of light regulation of AS

Alternative splicing patterns are determined by both *cis*-splicing regulatory elements and *trans*-acting splicing factor (52). Serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) are two well-known *trans*-splicing factors that either facilitate or restrain spliceosome assembly in response to environmental cues (53–56), and their expression or activity is regulated by light (28,57,58). Five splicing factors (*RS31*, *SR30*, *SR34a*, *SR34b* and *U2AF65a*) have been reported to participate in the phytochrome-mediated AS regulation (27,37). AS regulation of *AtRS31* is particularly essential for the response to changing level of light conditions in *Arabidopsis* (37). Additionally, *AtSR45a*, another SR-coding gene, suppresses the splicing efficiency by facilitating to form a bridge between 5' and 3' splice sites in response to high light stress (59). Given the widespread AS events affected by light are enriched in encoding mRNA processing (24) and splicing factors are significantly enriched among phytochrome-regulated genes in *Arabidopsis* (27), we posit that light-mediated AS is mostly regulated through the modulation of AS of RNA-processing genes themselves (Fig. 1). Most photoreceptors, including phytochromes, cryptochromes, *ZTL/LKP2/FKF1* and *UVR8*, are located in the nucleus (60–63), which may directly interact with the spliceosomes. It will be intriguing to investigate whether the components of photoreceptors can interact with the above splicing factors directly.

*RRC1* (reduced red-light responses in *cry1cry2* background 1) encodes a potential splicing factor that regulates the AS of

several SR genes in response to red light, such as *RS31* and *SR34b* (64). Splicing-defective mutant of *rrc1* is insensitive to red light in the phyB-mediated responses (64). However, another splicing factor mutant *SKIP* is hypersensitive to both red and blue light (46). Therefore, these inverse effects of splicing factors will prompt us to systematically determine the quantitative regulation of AS in response to light.

Aberrant photomorphogenic phenotypes are observed in defects of transcription factors (TFs), including B-box zinc finger (e.g. *BBX22*), basic helix–loop–helix (e.g. *PIF3*) and basic region/leucine zipper motif (e.g. *HYH*, *HY5*) TFs (65). In addition to splicing factors, TFs also affect AS by influencing the transcription elongation rate of RNA polymerase II (52,66,67). ChIP-seq peaks of *CCA1* include binding peaks near the promoter of several splicing-related genes, which suggests that *CCA1* might regulate AS indirectly by regulating splicing-related genes, such as *U11/U12-65K*, *GRP7* and *GRP8* (68). *SIC* regulates pre-mRNA metabolism and regulates the AS of circadian clock transcripts, including *LHY*, *CCA1*, *ELE3* and *PRR7* (69). The *sic*-associated clock impairment phenotypes required the presence of *CCA1* and *LHY* (69). However, the linkage among light-responsive TFs and the subsequent AS pattern is still not fully understood (Fig. 1). It is possible that light-regulated splicing factors, as well as transcription factors, act in coordination to regulate AS patterns in response to light (Fig. 1).

Regulation of AS is achieved by dynamic reciprocity between *trans*-splicing factor and *cis*-regulatory elements to define exon/intron boundaries and produce accurate splicing variants (70). There is no enriched motif around splicing sites from light-mediated AS events based on RNA-seq data (24), which might distinguish both positive and negative influences from different light-regulated splicing factors. Thus, it would be informative to analyze the *cis*-acting regulatory elements individually to identify both positive and negative regulatory elements. Cross-linking and immunoprecipitation, followed by high-throughput sequencing (CLIP-seq), are the most appropriate techniques to capture really binding motif of a single RNA-binding protein in plant (71). New breakthroughs are anticipated by combination of CLIP-seq with genetic overexpression or knockouts of splicing factors to clarify the molecular mechanism of specific splicing factors that are highly regulated by light.

### AS of photoreceptors and clock-related genes

As shown in Table 1, AS plays a key role in regulating photomorphogenesis and clock-related genes. For example, *PHYA* in *Arabidopsis* was annotated to include intron retention and produce an N-terminal truncated protein according to the TAIR10 annotations (72). In tomato, the *PHYA* gene is also regulated by AS which lend credence to the idea that it is a conserved mechanism (73). In addition to phytochromes, *CRY2* has one alternative accept site in exon 2 and produces different 5' UTR according to the annotation in TAIR10 (72) and ASIP database (8). AS variants on 5' UTR of *CRY2* may affect their mRNA stability or translatability. As mentioned above, the light regulation of genomewide AS switch is dependent, at least in part, on photosensory photoreceptors (27). On the other hand, these photosensory photoreceptor genes undergo the AS regulation; thus, it can form a feedback loop in response to light (Fig. 1). In addition to phytochromes and cryptochromes photoreceptors, other regulators of photomorphogenesis and circadian clocks such as

**Table 1.** Photomorphogenesis-related gene and circadian clock genes regulated by AS.

Gene	Locus	AS type	AS region	Light-regulated	References
<i>PHYA</i>	AT1G09570	IR; AltD	CDS	phy-regulated	(27)
<i>CRY2</i>	AT1G04400	AltA	5'-UTR	phy-regulated	(27,34)
<i>ZTL</i>	AT5G57360	IR	CDS	Unknown	(72)
<i>COP1</i>	AT2G32950	AltA	CDS	phy-regulated	(27,74)
<i>HY5</i>	AT5G11260	IR; AltD	CDS	2-h white light	(24,34)
<i>HYH</i>	AT3G17609	IR; AltA	CDS	phy-regulated	(27,77)
<i>LHY</i>	AT1G01060	IR; ES	5'-UTR/CDS	phy-regulated/2-h white light	(24,27)
<i>CCA1</i>	AT2G46830	IR; AltD	CDS	phy-regulated	(27,79)
<i>TOC1</i>	AT5G61380	IR	CDS	Unknown	(34)
<i>RVE8</i>	AT3G09600	AltA	CDS	phy-regulated/2-h white light	(24,27)
<i>JMJD5</i>	AT3G20810	IR	CDS	phy-regulated/2-h white light	(24,27)
<i>TIC</i>	AT3G22380	IR	CDS	phy-regulated/2-h white light	(24,27)
<i>CKB3</i>	AT3G60250	IR; AltA	CDS	phy-regulated/2-h white light	(24,27)
<i>PIF6</i>	AT3G62090	IR	CDS	Unknown	(93)

IR, intron retention; ES, exon skipping; AltD, alternative donor sites; AltA, alternative acceptor sites. The AS type of each gene is referenced to TAIR10 (72), ASIP (8) or AtRTD (34).

*COP1*, *HYH*, *HY5*, *LHY* and *CCA1* also undergo AS. It has been reported that *COP1* produces different AS isoforms (74). Overexpression of short isoform of *COP1* with truncated WD-40 repeat shows short hypocotyl and developed cotyledons in the dark (74), implying that AS of *COP1* may be relevant to the regulation of its physiological activities. Phytochromes and cryptochromes regulate light responses primarily through the transcriptional regulation of a great number of genes. Photoactivated phytochromes and cryptochromes inactivate COP1/SPA E3 ligase complex to regulate the abundance of two bZIP transcription factors, HY5 and HYH, which are the positive regulators in photomorphogenesis (75,76). Comparing with full-length HYH, the truncated HYH generated from the product of AS regulation, which lacks the COP1-interaction domain, is less susceptible to COP1-mediated degradation (77). *HY5* also includes intron retention and alternative donor in the coding region according to AtRTD2 annotation (34). The splicing isoforms of *LHY* can be recognized by NMD pathway (78). For another example, *CCA1* $\beta$  encoding truncated proteins interfere with the activity of functional protein *CCA1* $\alpha$  by competitively forming functional heterodimers (19).

### The function of AS alteration in response to light

The correlation between light and AS has been explored in a genome-wide scale by RNA-seq. However, investigation of biological function or specific splicing variants is time-consuming because there is no high-throughput experimental method to identify whether isoforms possess specific function *in vivo*. Even so, distinct functions of splicing isoforms induced by light have been identified in some cases (Fig. 1). For example, phytochromes induce splicing alteration of SPA3 and produce a truncated protein lacking in WD40 repeats (27), which is still able to interact with COP1 and form COP1/SPA complex but fails to bind to DAMAGE DNA-BINDING PROTEIN 1 (DDB1) to make up a multimeric E3 ubiquitin ligase (27). Another example is *CCA1*, which produces *CCA1* $\beta$  lacking the MYB domain. It works as a regulator by competitively inhibiting the DNA binding of functional *CCA1* $\alpha$  by forming *CCA1* $\alpha$ -*CCA1* $\beta$  heterodimers (19,79). It was also reported that splicing isoforms (mRNA3) of *AtRS31*, which possess PTC, showed increased expression in dark (37). However, the full-length splicing isoforms (mRNA1) encoding functional protein present decreased

expression (37). Finally, light-mediated AS produces proteins with different subcellular localization. For example, light signaling induces the expression of HPR2 located in the cytosol rather than HPR1 located in leaf peroxisomes (80). Given that AS is the major contributor to protein diversity, it is necessary to explore the function of splicing isoforms in a large scale. It will be interesting to investigate the photomorphogenic changes by overexpressing the light-induced isoforms to interrogate the functional information.

## CONCLUSIONS AND PERSPECTIVES

As shown above, light regulates AS mainly *via* photoreceptors or retrograde signals from chloroplast, while photoreceptors themselves are also under the regulation of AS to form a feedback loop. The regulation of light-mediated AS is mostly through the regulation of AS of splicing factors themselves. Light-mediated AS mainly confers its functional effects on dominant negative regulation, different subcellular localization or taking part in NMD pathway.

Recent observation reveals that mRNAs and protein abundances present a negative correlation upon illumination, suggesting diverse post-transcriptional regulation takes place in light response (81). In addition to AS, alternative polyadenylation (APA) and small RNAs, including miRNAs and siRNAs, are another post-transcriptional regulators in plants (82,83). MiRNAs are involved in photomorphogenesis according to studies on *HY5* (84), *HEN1* (85) and *AGO1* (86). The *ago 1* mutant displays light hypersensitive phenotype, suggesting that miRNAs act as negative regulators of photomorphogenesis (86). The mutation in *HY5* causes aberrant light-mediated phenotypes, which might result from the direct binding on the upstream of eight miRNA genes and subsequently affect the expression of miRNA target genes (84). Several animal studies confirm that miRNA regulates AS by targeting splicing regulator, such as miR-124 targeting PTBP1 (87), miR-133 targeting nPTB (88) and miR-222 targeting Rmb24 (89). In *Arabidopsis*, *SERRATE* plays roles in both pri-miRNA processing and mRNA splicing (90) and miRNAs can target AS region (91); thus, further evidences in future will extend our understanding of the interplay between miRNA and AS (Fig. 1).

Like AS, APA is also a widespread post-transcriptional regulator to generate transcript diversity in plants by changing the



coding region or the length of the 3'-UTR (71,92). Recent study shows that CCA1 has direct binding peak on the promoter region of *poly(A) binding protein 6* (68). However, the role of APA in response to light has never been reported. Does APA take part in light-regulated development, and if so, does it have dynamic interplay with either miRNA or AS? It may be expected that, taking full account of the coordinated influences above, most common post-transcriptional regulation will fully dissect the molecular mechanisms underlying photomorphogenic responses in plants.

**Acknowledgements**—The authors thank Dr. Marcelo J. Yanovsky and Yoshito Oka for critical reading of the manuscript. This work was supported by the National Key Research and Development Program of China (2016YFD0600106) and the National Natural Science Foundation of China Grant (Grant No. 31570674 to L.G.).

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